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PHYSIOLOGY RESEARCH ON MUSCLE

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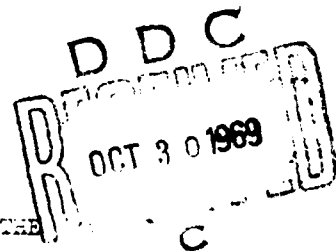
PRINGLE, J.W.S. & CULLEN, M.J.

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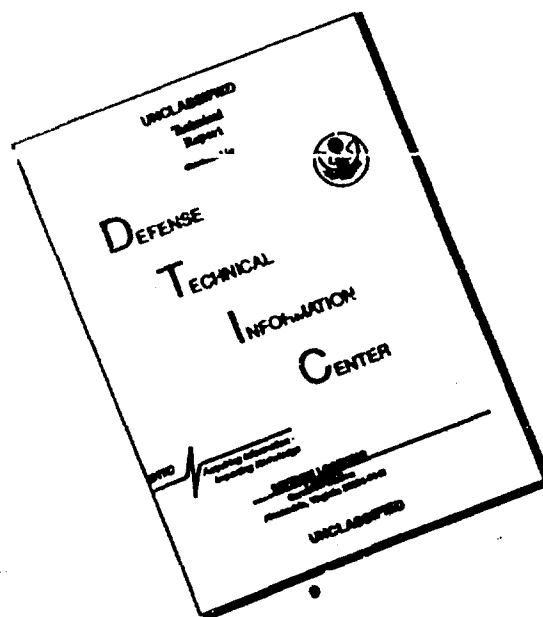
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OBJECTIVES

This Grant was made to permit the continuation for one year of the experimental breeding programme of giant water bugs started under Grant AF EOAR 66-52. It provided the salary of a research assistant, Mr M.J. Cullen, funds for the purchase of tanks and cages, and part of the cost of services necessary in the tropical room.

GENERAL SUMMARY OF RESULTS ACHIEVED

Details of the work carried out are given in the appended report by Mr M.J. Cullen. The experience gained in the period of the two grants has made it possible to finalise the design of the improved tropical climate room in the new laboratory now under construction. A handbook giving instructions for the maintenance and breeding of giant water bugs will be produced after one year's experience in the new laboratory.

DETAILED REPORT

M.J. Cullen

Fittings

The fluorescent tube lighting arranged in frames (as described in the Final Scientific Report for Grant AF EOAR 66-52, July 1968) has proved highly satisfactory in the present tropical room. The tubes have a long life and provide enough light to support healthy plant growth. A similar lighting system is therefore going to be used in the new tropical room, but, as a far greater intensity of lighting is going to be used and consequently much more heat produced, the frames will be

perforated to allow an outward flow of air from the room and into the extractor ducts of a ventilation system, so providing direct cooling of the lighting system. The frames in the present room are slung on chains from the ceiling so that their height over the bug tanks can be varied. This arrangement has in fact proved to be unnecessary and in the new room the frames will be at a fixed level, at about 80 inches from floor level.

The breeding chamber in the new tropical room will be built around one lighting frame instead of being illuminated separately by three 400 watt bulbs as at present (see Final Scientific Report for Grant AF EOAR 66-52, July 1968). Besides the fluorescent tube day lighting there will be a low wattage 'moon light' to simulate tropical night conditions. It has been established that giant water bugs confine most of their flight activity to a few days around the full moon (see Interim Report for Grant AF EOAR 66-52, July 1967) and it would be of great interest if they could be induced to fly naturally in the laboratory. In the new room there will be two red lamps which will allow observation of any nocturnal activity. Red light is thought to be invisible to most insects.

For most of the year, the lighting system in the present tropical room allows the humidity to follow a diurnal cycle similar to that found in a tropical climate. Humidity reaches a peak, approaching 100% RH before the lights switch on in the morning, after which the RH declines to about 50%. After the lights switch off and

the temperature of the air falls below that of the water, the humidity again rises. During the winter, however, when the temperature of the room is controlled by a supplementary heating system and not by the lights a uniform temperature is maintained throughout the 24 hours and the RH stays at a low level of about 40%. To prevent this happening in the new tropical room, a humidifier is being positioned in the ventilation system so that water vapour is added to the heated returning air. (In fact it should not be necessary for the humidifier to operate very often as even in the coldest part of the year the lights will probably be a sufficient source of heat).

A drawback of the present tropical room is that during the summer there is only a small loss of heat from the room and the temperature is raised above the ideal level. The optimal mean temperature required is around 23°C (see Progress Report No. 1, on Grant EOOAR-68-0032). During June and July of this year the temperature has regularly risen above 35°C (95°F). This will not occur in the new tropical room as the ventilation system, mentioned above, will be thermostatically controlled so that the temperature is maintained between upper and lower limits of 35°C and 15°C respectively. The cooling will be by an inward flow of air from outside. Too high a temperature is undesirable as it greatly increases the demands upon the food supply by the bugs, and the food fish themselves are rendered sterile by prolong exposures to temperatures above 28°C .

The lowest room temperature in the tropical room is reached in the early morning before the lighting is switched on. This has seldom dropped below 20°C (in the winter thermostatically controlled electrically heated tubes are turned on when the room temperature falls below 20°C) and a low room temperature has probably never been responsible for any great mortality amongst the bugs. For the new room should the temperature drop below, say, 20°C (this temperature can be altered to give the most favourable diurnal fluctuation) a thermostatically controlled heater in the ventilation system will automatically heat air recirculated to the room. The only serious cases of death caused by cold have been when the drip feed of water to the bug tanks has become uncontrolled the large volumes of cold water have passed through the tanks. In the new tropical room in the event of any electrical failure the water supply will be automatically switched off so there will be no danger of cold water flowing through the tanks.

At present the water passing through the tanks containing the bugs comes directly from the mains supply. At certain times of the year this water becomes rich in nitrates and other inorganic and organic ions (perhaps from the run-off from artificially fertilised agricultural land) which encourage the growth of blue green algae in the tanks. This can form a slime on the water surface which in bad cases can clog the respiratory system of the bugs and cause their death. This possibility will be guarded against in the new tropical room by

the use of rainwater to supply the slow feed to the tanks. A rainwater storage tank holding 2,500 gallons will be situated on the floor level above that of the tropical room. In the present room each of the 2 tiers of five 25 gallon tanks has a water inflow rate of $2\frac{1}{2}$ gallons an hour, making a total consumption of 120 gallons a day. That of the new room will be double this, so the storage tank will hold about 10 days' supply of water. In the rare event of there being more than 10 days without rainfall, the tropical room will be switched over to a supply of deionised water which, like rainwater, will not support the growth of the blue green algae. The fish breeding tanks, in which the presence of blue green algae is not harmful will be supplied with mains water.

Livestock

The breeding of Limnogeton fieberi is continuing (see Progress Report No. 2, for Grant EOAR-68-0032). Of the small batch of eleven eggs laid 16.4.69, eight successfully hatched 13 days later and four of these are now in the fifth nymphal instar. Recently several more egg batches have been obtained. A batch of eggs was laid on 11.6.69 and some of this brood are now in third instar. Another batch, laid 27.6.69 are now in the first and second instar, and one laid 4.7.69 are all still in the first instar. Two more one week old batches of eggs have not yet hatched. An interesting feature of the rearing of this bug is that mortality has been far less in nymphs kept at

mean temperatures of $< 20^{\circ}\text{C}$ than in those kept at 30°C . Of 25 nymphs kept in the tropical room 13 died in the first two weeks of life whereas of 15 kept in my room at a normal English summer room temperature only one has died. Both groups have been given a plentiful supply of small snails as food. The high mortality amongst those nymphs kept in the tropical room might have been due to the high temperature ($35\text{--}40^{\circ}\text{C}$) reached in there this summer. This temperature is probably higher than that of their natural habitat (P.L. Miller - personal communication). The species of aquatic snail used as food for Limnogeton are Planorbis corneus and Limnaea peregra. Limnogeton is not cannibalistic so the nymphs are kept several to a tank.

Despite being exposed to a variety of conditions Lethocerus has still not oviposited normally in the laboratory. Parameters which have been varied include day length, light intensity, air and water temperature, relative humidity and the biological environment (vegetation and prey). Apart from their habit of depositing eggs in the water the behaviour of the bugs appears normal and lively. A factor which has not yet been altered is the hardness of the water. As Lethocerus is found in still rather than running water it might favour water with a high organic content with $\text{pH} < 7$. The water supply used for the breeding chamber comes from the mains supply and is very hard. It might be worthwhile therefore to change the water in the breeding chamber for rain, distilled or chemically softened water. An argument

against this having any effect is that Limnogeton, Hydrocyrius and Belostoma which are also still water insects breed freely in water originating from the Oxford water supply. I also have intentions of trying to breed other species of Lethocerus and have written to Dr D.S. Smith in Florida to find out whether he can supply any L. griseus or L. americanus. These are temperate as well as tropical species for which it might be easier to find acceptable conditions for normal oviposition.

Hydrocyrius columbiae continues to breed successfully (see Progress Report No. 1 for Grant EOAR-69-0032). It sometimes happens that more of these arrive in one parcel from Uganda than can be contained in the available individual cages. The excess bugs are temporarily put in the largest fish-breeding tank which is well planted with vegetation. These bugs sometimes breed there and males carrying egg batches appear. Two broods have been started in this way and a third batch of eggs^{was} allowed to hatch in the tank as facilities for dealing with another set of nymphs were not available at the time. These nymphs have been allowed to continue living 'free-range' in the large tank, and, despite a certain amount of cannibalism, some of them are thriving.

As was pointed out in Progress Report No. 1, a drawback of relying on Hydrocyrius as an experimental animal is the long period of 4-5 months required for them to differentiate fully mature flight

muscles. (Some have been known to pass into the reproductive phase in the laboratory without every maturing their muscles). Coupled with this problem is the tendency for the flight muscles of Hydrocyrius to degenerate soon after the animal has dispersed. Immature and degenerate muscles of Hydrocyrius have now been looked at electron-microscopically and their fine structure compared with that of the normal mature flight muscles.

Dorsal longitudinal muscle taken from an adult bug two weeks after its final ecdysis, bears little resemblance to that of the fully mature and flight-worthy insect. The bulk of the fibre seems to be occupied by nuclei and a granular matrix. The myofibrils are of very small diameter (0.2μ) and contain only 15-20 myosin filaments (and 45-60 actin filaments) compared with 5,000 in the mature muscle. The filaments have the arrangement and spacing of the normal muscle and the length of the rudimentary sarcomere corresponds to that of the relaxed muscle. Mitochondria are very rare. The tracheoles are very obvious, because like the nuclei they are concentrated in a small volume of fibre. Tubules of the T system forming a network between the muscle membrane and the tracheolar inpushings are very obvious. Degenerate muscle bears a resemblance to immature muscle. The nuclei and tracheoles are again greatly concentrated and there is a similar matrix which contains many small granules which may be of glycogen. Between the shrunken fibres lies a lot of amorphous material, presumably

produced from the breakdown of the contractile material. The fibrils are greatly reduced in diameter ($0.4-0.8\mu$) but the sarcomeres are of normal relaxed length. The mitochondria are rounded off, reduced in size and in various stages of vacuolation. It seems likely that as degeneration proceeds further, all the filaments of the fibrils break down and the mitochondria disappear leaving little besides the tracheoles and the nuclei within the muscle fibre membrane.

A small number of Belostoma malkini are being kept 'free-range' in one of the fish breeding tanks. These breed and regularly produce batches of eggs, but, because the flight muscles of Belostoma are small and of limited use, no special effort is made to rear the nymphs. Some of these do metamorphose naturally however, so a reserve population is maintained. It is conceivable that in the future Belostoma (probably the easiest belostomatid to rear) may be required for salivary gland, scent gland, muscle maturation/degeneration or other biological investigations, in which case it will be useful to be able to build up stocks from the small reserve population.

The present stock of bugs is constituted thus:

<u>Limnogeton fieberi</u> adults	28
nymphs	26
<u>Lethocerus cordofanus</u>	24
<u>Lethocerus maximus</u>	2
<u>Hydrocyrius columbiae</u> adults	30
nymphs	25
<u>Belostoma malkini</u>	10
Total	145

As an appendix, a domestic "experiment" with Belostoma might be mentioned. The author at home keeps tropical aquaria, one of which recently became over-run with Planorbis. Two one-day old Belostoma nymphs were added to this tank and within 7 weeks the tank had been cleared of snails and the bugs had metamorphosed to adults. This is about the shortest recorded time for the metamorphosis of any belostomatid and underlines the importance of an abundant food supply.

Summary

(1) The lighting system of fluorescent tubes arranged in a number of separate reflecting aluminium frames has proved to be perfectly suitable and will be used in the new tropical room with little modification.

(2) To prevent too high a room temperature being produced by

the lighting (as happens in summer in the present tropical room) the new tropical room will include a ventilation system which will extract the hot air through the lighting frames. A heater and humidifier will be incorporated in the return sections of the ventilation system and will be operated in the event of the room temperature falling below 20°C.

(3) Blooms of blue green algae periodically occur in the bug tanks in the present tropical room which is fed with water direct from the mains supply. To guard against this the tanks in the new tropical room will be supplied with rain water which will not support the growth of this algae.

(4) Despite the use of different regimes of day length, light intensity and temperature it has not yet been possible to induce Lethocerus to oviposit normally. It is planned to carry on experimenting by altering the pH of the water and by trying other species of Lethocerus.

(5) Limnogeton and Hydrocyrius require little inducement to breed. Hydrocyrius however takes 4-5 months to mature its flight muscles and is then likely to degenerate them during its reproductive phase. Limnogeton metamorphoses quicker but it is not yet known how long flight muscle maturation takes in this bug. A lower mortality occurs in the rearing of Limnogeton when the nymphs are kept at about 20°C than when they are kept at 30°C.

(6) A small reserve reproductive population of Belostoma is being maintained.

(7) The functioning of the tropical room as a place for keeping the adult bugs sent from abroad alive and well for as long as possible has been greatly improved over the last two years. This has largely been brought about by increasing the stocks of suitable prey and by finding an optimum ambient temperature range. Bugs can now be kept lively and healthy for periods of many months.

Reference

Cullen, M.J. (1969) The Biology of Giant Water Bugs (Hemiptera, Belostomatid e) in Trinidad. Proc. Roy. ent. Soc. Lond. A, 44, (in press).

Reports

Progress Report No. 1	12 March 1969
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